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(54) Title: HUMAN FGF GENE AND GENE EXPRESSION PRODUCTS

(57) Abstract

This invention relates to human fibroblast growth factor (FGF–X), and to variants thereof and to polynucleotides encoding FGF–X.The invention also relates to diagnostic and therapeutic agents related to the polynucleotides and proteins, including probes and antibodies.

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HUMAN FGF GENE AND GENE EXPRESSION PRODUCTS

TECHNICAL FIELD

The present invention relates to novel nucleic acid sequences encoding a member of the fibroblast growth factor (FGF) family, and to polypeptides encoded by the nucleic acid sequences.

BACKGROUND OF THE INVENTION

This invention provides a gene of the fibroblast growth factor (FGF) family and protein encoded by the gene. The invention is directed to mRNA expressed by human cells, polynucleotides having coding regions corresponding to the mRNA, protein products of the polynucleotides and polypeptide products of mRNA, and biological function of the polypeptides and proteins.

At least 17 members of the FGF family have been identified, and are discussed in, for example, Ohuchi et al., *Development 124*:2235-2244 (1994); Ghosh et al., *Cell Growth and Differentiation 7*:1425-1434 (1996); Gemel et al., *Genomics 35*:253-257 (1996); Crossley et al., *Development 121*:439-451 (1995); and Crossley et al., *Cell 84*:127-136 (1996).

SUMMARY OF THE INVENTION

The present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising at least eight contiguous nucleotides from nucleotide 18 to 461 of SEQ ID NO:1;

(b) a polynucleotide that encodes a variant of the polypeptide encoded by (a); and

(c) a polynucleotide encoding a protein expressed by a polynucleotide having the sequence of SEQ ID NO:1.

The invention further provides for the use of the isolated polynucleotides or fragments thereof as diagnostic probes or as primers.

The present invention also provides a composition comprising a polypeptide, wherein said polypeptide is selected from the group consisting of:

(a) a polypeptide comprising at least 6 contiguous amino acids encoded by SEQ ID NO:1 in the region of nucleotide 18 to 461;

(b) a polypeptide encoded by a polynucleotide comprising SEQ ID NO:1; and

(c) a variant of the protein (a) or (b).

In certain preferred embodiments of the invention, the polynucleotide is operably linked to an expression control sequence. The invention further provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with the polynucleotide sequence. The invention also provides full-length cDNA and full-length polynucleotides corresponding to SEQ ID NO:1.

Protein and polypeptide compositions of the invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody that specifically reacts with such protein or polypeptide are also provided by the present invention.

In a preferred embodiment, pulmonary system cells when treated with FGF-X of the invention proliferate, survive injury, have at least a limited self regeneration capacity, or can undergo lineage restriction in response to the local environment.

The invention also provides for the production of large amounts of otherwise minor cell populations of cells to be used for generation of cDNA libraries for the isolation of rare molecules expressed in the precursors cells or progeny; cells produced by treatment may directly express growth factors or other molecules, and conditioned media is screened in assays for novel activities.

The invention further provides for the isolation, self-renewal and survival of mammalian stem cells and the differentiation of their progeny.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a Northern blot showing expression of FGF-X mRNA in trachea, and little or no expression in the other tissues tested.

Figure 2 illustrates the relationship between known FGF proteins and FGF-X.

Figure 3 illustrates FGF-X mRNA expression in human brain, lung and thyroid.

Figure 4 provides codon usage for yeast. The first field of information on each line of the table contains a three-letter code for an amino acid. The second field contains an unambiguous codon for that amino acid. The third field lists the number of occurrences of that codon in the genes from which the table is compiled. The fourth field lists the expected number of occurrences of that codon per 1,000 codons in genes whose codon usage is identical to that compiled in the codon frequency table. The last field contains the fraction of occurrences of the codon in its synonymous codon family.

Figure 5 provides codon usage for Drosophila.

Figure 6 provides codon usage for E. coli.

DETAILED DESCRIPTION OF THE INVENTION

Because of their potent activities for promoting growth, proliferation, survival and differentiation of a wide variety of cells and tissue types, FGFs continue to be pursued as therapeutic agents for a number of different indications, including wound healing, such as musculo-skeletal conditions, for example, bone fractures, ligament and tissue repair, tendonitis, bursitis, etc.; skin conditions, for example, burns, cuts, lacerations, bed sores, slow healing ulcers, etc.; tissue protection and repair during myocardial infarction and ischemia, in the treatment of neurological conditions, for example, neuro-degenerative disease, neuronal injury, and stroke, in the treatment of eye disease, including macular degeneration, retinitis pigmentosa, and the like.

The fibroblast growth factor (FGF) proteins identified to date belong to a family of signaling molecules that regulate growth and differentiation of a variety of cell types. The significance of FGF proteins to human physiology and pathology relates

in part to their key roles in embryogenesis, in blood vessel development and growth, and in bone growth. *In vitro* experiments have demonstrated a role for FGF in regulating cell growth, division or differentiation of endothelial cells, vascular smooth muscle cells, fibroblasts, cardiac and skeletal myocytes, and various nervous system cells. Members of the FGF family and their biological roles are described in Crossley et al., *Development 121*:439-451 (1995); Ohuchi et al., *Development 124*:2235-2244 (1997); Gemel et al., *Genomics 35*:253-257 (1996); and Ghosh et al., *Cell Growth and Differentiation 7*:1425-1434 (1996).

FGF proteins are also significant to human health and disease because of a role in cancer cell growth. For example, FGF-8 was identified as an androgen-induced growth factor in breast and prostate cancer cells. (Tanaka et al., *FEBS Lett. 363*:226-230 (1995) and *P.N.A.S. 89*:8928-8932 (1992)).

A member of the FGF family is described here, wherein the FGF protein is expressed at high levels in trachea. A polynucleotide encoding FGF of the invention has the sequence as shown in SEQ ID NO:1. The polynucleotide was identified as encoding a member of the FGF family by the conserved regions throughout the amino acid sequence and by the regions of homology shared by the polynucleotide and genes encoding known FGF proteins.

The inventors believe that FGF-X is a previously unidentified member of the human FGF family. To date, 18 human FGF proteins have been identified. In most cases, homologous proteins in other mammals, particularly mice and rats, have also been identified. The human proteins vary to different degrees in terms of amino acid sequence, receptor specificity, tissue expression patterns, and biological activity.

FGF-1 and FGF-2 are also referred to as acidic and basic fibroblast growth factor, respectively. These proteins are modulators of cell proliferation, cell motility, differentiation, and cell survival. Their target cells include cells of the ectoderm, mesoderm, and endoderm.

FGF-3 is a target for activation by the mouse mammary tumor virus (Dickson et al., 1991, *Ann. N.Y. Acad. Sci. 638*:18-26). FGF-4, FGF-5 and FGF-6 are oncogene products (Yoshida et al., 1991, *Ann. N.Y. Acad. Sci. 638*:27-37; Golfarb et al.,

1991, *Ann. N.Y. Acad. Sci. 638*:38-52; Carlier et al., 1991, *Ann. N.Y. Acad. Sci. 638*:53-61). FGF-7, FGF-8 and FGF-9 are mitogens for cells in culture (Aaronson et al., 1991, *Ann. N.Y. Acad. Sci. 638*:62-77; Taneka et al., 1993, *Proc. Nat'l. Acad. Sci. 89*:8928-8932; Miyamoto et al., 1993, *Mol. Cell. Biol. 13*:4251-4259).

FGF-10 - FGF-14 have been identified and described (Yamasaki et al., 1996, *J. Biol. Chem. 271*:15918-15921; Smallwood et al., 1996, *Proc. Nat'l Acad. Sci. 93*:9850-9857). Human FGF-15 is described in U.S. Patent No. 5,773,252 (Greene et al.). FGF-16 and FGF-17 were identified from rat heart and embryos (Miyake et al., 1998, *Biochem. Biophys. Res. Commun. 243*:148-152; Hoshikawa et al., 1998, *Biochem. Biophys. Res. Commun. 244*:187-191). FGF-18 was described by Hu et al., 1998, *Mol. Cell. Biol. 18*:6063-6074. Mouse FGF-18 was described in Deisher et al., WO 98/16644.

The present FGF-X differs in sequence from all the human FGF proteins described to date. FGF-X shares some homology with mouse FGF-15 (Figure 2). However, the same degree of homology does not exist between FGF-X and the molecule identified as human FGF-15.

As the above brief discussion illustrates, the knowledge about the roles played by various FGF proteins continues to grow, but is by far incomplete.

The present invention adds to this knowledge by disclosing that the FGF of SEQ ID NO:1 is expressed in human lung, and is particularly highly expressed in trachea, and may play a role in development of and recovery from a variety of pulmonary conditions, such as loss of pulmonary, bronchia or alveolar cells or function, healing of pulmonary or bronchial wounds, pulmonary infarction, emphysema/chronic obstructive pulmonary disease, asthma, sequelae of infectious or autoimmune disease, sequelae of pulmonary arterial or venous hypertension, pulmonary fibrosis, pulmonary disease of immaturity, and cystic fibrosis.

The present invention also adds to this knowledge by disclosing that the FGF of SEQ. ID:1 is detected in human brain and thyroid tissues, and in a human parathyroid tumor. FGF-X may therefore play a role in the development and recovery from a variety of central nervous system diseases, including peripheral neuropathy,

amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, ischemic stroke, acute brain injury, acute spinal chord injury, nervous system tumors, multiple sclerosis, peripheral nerve trauma or injury, exposure to neurotoxins, metabolic diseases such as diabetes or renal dysfunctions and damage caused by infectious agents. Where the cellular degeneration involves bone marrow cell degeneration, the diseases include, but are not limited to disorders of insufficient blood cells such as, for example, leukopenias including eosinopenia and/or basopenia, lymphopenia, monocytopenia, neutropenia, anemias, thrombocytopenia as well as an insufficiency of stem cells for any of the above. The above cells and tissues can also be treated for depressed function.

Further, FGF-X may therefore play a role in the development and recovery from a variety of thyroid diseases, including thyroid tumors and cancer, autoimmune and inflammatory thyroid disease, hypothyroidism and hyperthyroidism/Grave's disease. FGF-X may play a role in the development and recovery from parathyroid diseases, including parathyroid tumors and cancer, hyperparathyroidism and hypoparathyroidism.

The invention therefore is based upon the identification, isolation and sequencing of a new fibroblast growth factor (FGF-X).

Reference to FGF-X herein is intended to be construed to include growth factors of any origin that are substantially homologous to and that are biologically equivalent to the FGF-X characterized and described herein. Such substantially homologous growth factors may be native to any tissue or species and, similarly, biological activity can be characterized in any of a number of biological assay systems.

The term "biologically equivalent" is intended to mean that the compositions of the present invention are capable of demonstrating some or all of the same growth properties in a similar fashion, not necessarily to the same degree as the FGF-X isolated as described herein or recombinantly produced human FGF-X of the invention.

By "substantially homologous" it is meant that the degree of homology of human FGF-X to FGF-X from any species is greater than that between FGF-X and any previously reported member of the FGF family.

Sequence identity or percent identity is intended to mean the percentage of same residues between two sequences, referenced to human FGF when determining percent identity with non-human FGF-X, referenced to FGF-X when determining percent identity with non-FGF-X growth factors, when the two sequences are aligned using the Clustal method (Higgins et al, Cabios *8*:189-191, 1992) of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, WI). In this method, multiple alignments are carried out in a progressive manner, in which larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues in the alignment, determined from a residue weight table representing the probability of a given amino acid change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment=10; gap length penalty for multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise alignment=5. The residue weight table used for the alignment program is PAM250 (Dayhoff et al., in Atlas of Protein Sequence and Structure, Dayhoff, Ed., NDRF, Washington, Vol. 5, suppl. 3, p. 345, 1978).

Percent conservation is calculated from the above alignment by adding the percentage of identical residues to the percentage of positions at which the two residues represent a conservative substitution (defined as having a log odds value of greater than or equal to 0.3 in the PAM250 residue weight table). Conservation is referenced to human FGF-X when determining percent conservation with non-human FGF-X, referenced to FGF-X when determining percent conservation with non-FGF-X

growth factors. Conservative amino acid changes satisfying this requirement are: R-K; E-D, Y-F, L-M; V-I, Q-H.

The invention provides FGF-X proteins or variants thereof having one or more polymers covalently attached to one or more reactive amino acid side chains. By way of example, not limitation, such polymers include polyethylene glycol (PEG), which can be attached to one or more free cysteine sulfhydryl residues, thereby blocking the formation of disulfide bonds and aggregation when the protein is exposed to oxidizing conditions. In addition, pegylation of FGF-X proteins and/or muteins is expected to provide sucy improved properties as increased half-life, solubility, and protease resistance. FGF-X proteins and/or muteins may alternatively be modified by the covalent addition of polymers to free amino groups such as the lysine epsilon or the N-terminal amino group. Preferred cysteines and lysines for covalent modification will be those not involved in receptor or heparin binding. In both human and rat FGF-X, the heparin binding site comprises amino acids 170-186. It will be apparent to one skilled in the art that the methods for assaying FGF-X biochemical and/or biological activity may be employed in order to determine if modification of a particular amino acid residue affects the activity of the protein as desired.

It may be advantageous to improve the stability of FGF-X by modifying one or more protease cleavage sites. Thus, the present invention provides FGF-X variants in which one or more protease cleavage site has been altered by, for example, substitution of one or more amino acids at the cleavage site in order to create as FGF-X variant with improved stability. Such improved protein stability may be beneficial during protein production and/or therapeutic use.

Suitable protease cleavage sites for modification are well known in the art and likely will vary depending on the particular application contemplated. For example, typical substitutions would include replacement of lysines or arginines with other amino acids such as alanine. The loss of activity, such as receptor binding or heparin binding, can be tested for as described herein.

FGF-X can also include hybrid and modified forms of FGF-X including fusion proteins and FGF-X fragments and hybrid and modified forms in which certain

amino acids have been deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylations so long as the hybrid or modified form retains the biological activity of FGF-X. By retaining the biological activity, it is meant that neuronal survival is promoted, although not necessarily at the same level of potency as that of the FGF-X isolated as described herein or that of the recombinantly produced human FGF-X. Fusion proteins can consist of the FGF-X of the invention or fragment thereof and a signal sequence of a heterologous protein to promote secretion of the protein product.

Fusion proteins comprising FGF-X or a biologically active or antigenic fragment thereof can be produced using methods known in the art. Such fusion proteins can be used therapeutically or can be produced in order to simplify the isolation and purification procedures. Histidine residues can be incorporated to allow immobilized metal affinity chromatography purification. Residues EQKLISEEDL contain the antigenic determinant recognized by the myc monoclonal antibody and can be incorporated to allow myc monoclonal antibody-based affinity purification. A thrombin cleavage site can be incorporated to allow cleavage of the molecule at a chosen site; a preferred thrombin cleavage site consists of residues LVPRG. Purification of the molecule can be facilitated by incorporating a sequence, such as residues SAWRHPQFGG, which binds to paramagnetic streptavidin beads. Such embodiments are described in WO 97/25345, which is incorporated by reference.

The invention also includes fragments of FGF-X. Preferred fragments of SEQ ID NO:2 include: amino acids from about 1 to about 147; amino acids from about 2 to about 147; amino acids from about 56 to about 71; and amino acids from about 75 to about 89. Such fragments can be prepared from the protein by standard biochemical methods or by expressing a polynucleotide encoding the fragment.

The sequence of a nucleic acid comprising at least 12 contiguous nucleotides of SEQ ID NO:1, preferably the entire sequence of SEQ ID NO:1, is not limited and can be any sequence of A, T, G, and/or C (for DNA) and A, U, G, and/or C (for RNA) or modified bases thereof, including inosine and pseudouridine. The choice

of sequence will depend on the desired function and can be dictated by coding regions desired, the intron-like regions desired, and the regulatory regions desired. Where a sequence of SEQ ID NO:1 is within the nucleic acid, the nucleic acid obtained is referred to herein as a polynucleotide comprising the sequence of SEQ ID NO: 1. The invention further provides fragments of the disclosed polynucleotide sequence. Preferred fragments are 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 400, 410, 420, 425, 430, 435, 438, 441, 450, 500, 550, 600, 625, 650, 700, 750, 800, 850, 900, 1000, 1100, 1200, 1300, 1400, 1450, or 1500 contiguous nucleotides of SEQ ID NO:1.

Included within the scope of the invention are polynucleotides, including DNA and RNA, with 80% homology to SEQ ID NO:1; preferably at least 85% homology, more preferably at least 90% homology, most preferably 95% homology. Polynucleotides with 96%, 97%, 98%, and 99% homology to SEQ ID NO:1 are also included. Percent homology is calculated using methods known in the art. A non-limiting example of such a method is the Smith-Waterman homology search algorithm as implemented in MPSRCH program (Oxford Molecular), using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

One or more additional heparin binding sites can be added to FGF-X by, for example, expressing DNA encoding FGF-X wherein the codons corresponding to residues comprising the heparin binding site are inserted at the desired position(s) in the reading frame. DNA encoding FGF-X with altered receptor binding can likewise be produced. For example, it may be desirable to alter receptor specificity of FGF-X by substituting the receptor binding regions of a different FGF for that of FGF-X.

FGF-X can also include hybrid and modified forms of FGF-X including fusion proteins and FGF-X fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylations so long as the hybrid or modified form retains the biological activity of FGF-X. By retaining the biological activity, it is meant that FGF-X induces mitogenesis, promotes cell survival, or induces differentiation of endothelial

cells, granulosa cells, smooth muscle cells, pulmonary cells, fibroblasts, cells of the central nervous system, chondrocytes, or epithelial cells, although not necessarily at the same level of potency as that of the FGF-X isolated as described herein or that of the recombinantly produced human FGF-X.

It will be recognized in the art that some amino acid sequence of the FGF-X of the invention can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there are critical areas on the proteins that determine activity. In general, it is possible to replace residues that form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein. The replacement of amino acids can also change the selectivity of binding to cell surfacc receptors. Ostade et al., *Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the polypeptides of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

Thus, the invention further includes variations of the FGF-X of the invention which show substantial FGF-X activity. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990).

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the polypeptides of the invention. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et al., *Clin. Exp. Immunol. 2*:331-340 (1967); Robbins et al., *Diabetes*

36:838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

Amino acids in the FGF-X of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or in vitro, or in vitro proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., *J. Mol. Biol. 224*:899-904 (1992) and de Vos et al. Science 255:306-312 (1992)).

Changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein. Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of substitutions for FGF-X will not be more than 50, 40, 30, 25, 20, 15, 10, 5 or 3.

Also included within the meaning of substantially homologous is any FGF-X which may be isolated by virtue of cross-reactivity with antibodies to FGF-X described herein or whose encoding nucleotide sequences including genomic DNA, mRNA or cDNA may be isolated through hybridization with the complementary sequence of genomic or subgenomic nucleotide sequences or cDNA of FGF-X herein or fragments thereof. It will also be appreciated by one skilled in the art that degenerate DNA sequences can encode human FGF-X and these are also intended to be included within the present invention as are allelic variants of FGF-X.

Growth factors are thought to act at specific receptors. According to the invention, FGF-X and as yet unknown members of this family of growth factors act through specific receptors having distinct distributions as has been shown for other growth factor families.

A preferred FGF-X of the present invention is identified and isolated in purified form as described. Also preferred is FGF-X prepared by recombinant DNA technology. By "pure form" or "purified form" or "substantially purified form" it is meant that an FGF-X composition is substantially free of other proteins which are not FGF-X.

Recombinant human FGF-X may be made by expressing the DNA sequences encoding FGF-X in a suitable transformed host cell. Using methods well known in the art, the DNA encoding FGF-X may be linked to an expression vector, transformed into a host cell and conditions established that are suitable for expression of FGF-X by the transformed cell.

Any suitable expression vector may be employed to produce recombinant human FGF-X such as expression vectors for use in insect cells. Baculovirus expression systems can also be employed.

The present invention includes nucleic acid sequences including sequences that encode human FGF-X. Also included within the scope of this invention are sequences that are substantially the same as the nucleic acid sequences encoding FGF-X. Such substantially the same sequences may, for example, be substituted with codons more readily expressed in a given host cell such as E. coli according to well known and standard procedures. Such modified nucleic acid sequences are included within the scope of this invention.

Specific nucleic acid sequences can be modified by those skilled in the art and, thus, all nucleic acid sequences that code for the amino acid sequences of FGF-X can likewise be so modified. The present invention thus also includes nucleic acid sequence which will hybridize with all such nucleic acid sequences--or complements of the nucleic acid sequences where appropriate--and encode a polypeptide having the cell survival promoting activities disclosed herein. The present invention also includes nucleic acid sequences that encode for polypeptides that have neuronal survival promoting activity and that are recognized by antibodies that bind to FGF-X. A preferred method for raising antibody is described in Example 2, but the whole protein or fragments thereof can also be used to raise suitable antibodies.

The present invention also encompasses vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the invention. This invention also includes host cells--of any variety--that have been transformed with vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the present invention.

Methods are also provided herein for producing FGF-X. Preparation can be by isolation from conditioned medium from a variety of cell types so long as the cell type produces FGF-X. A second and preferred method involves utilization of recombinant methods by isolating or obtaining a nucleic acid sequence encoding FGF-X, cloning the sequence along with appropriate regulatory sequences into suitable vectors and cell types, and expressing the sequence to produce FGF-X.

Although FGF-X has been described on the basis of its expression in trachea, this factor is likely to be expressed in other tissues of the pulmonary system as well, as shown by expression in whole lung extracts..

It is also likely that FGF-X will act on cells to promote their survival, growth or function. This expectation is based upon the activity of known growth factors. Members of the FGF family act on many cell types of different function and embryologic origin.

The present invention also includes therapeutic or pharmaceutical compositions comprising FGF-X in an effective amount for treating patients with cellular degeneration and a method comprising administering a therapeutically effective amount of FGF-X. These compositions and methods are useful for treating a number of degenerative diseases, as well as recovery from a variety of pulmonary conditions, such as loss of pulmonary, bronchia or alveolar cells or function, healing of pulmonary or bronchial wounds, pulmonary infraction, emphysema/chronic obstructive pulmonary disease, asthma, sequelae of infectious or autoimmune disease, sequelae of pulmonary arterial or venous hypertension, pulmonary fibrosis, pulmonary disease of immaturity, and cystic fibrosis.

In certain circumstances, it may be desirable to modulate or decrease the amount of FGF-X expressed. Thus, in another aspect of the present invention, FGF-X anti-sense oligonucleotides can be made and a method utilized for diminishing the level of expression of FGF-X by a cell comprising administering one or more FGF-X anti-sense oligonucleotides. By FGF-X anti-sense oligonucleotides reference is made to oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary nucleic acid sequence involved in the expression of FGF-X such that the expression of FGF-X is reduced. Preferably, the specific nucleic acid sequence involved in the expression of FGF-X is a genomic DNA molecule or mRNA molecule that encodes FGF-X. This genomic DNA molecule can comprise regulatory regions of the FGF-X gene, or the coding sequence for mature FGF-X protein. The term complementary to a nucleotide sequence in the context of FGF-X antisense oligonucleotides and methods therefor means sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, *i.e.*, under physiological conditions. The FGF-X antisense oligonucleotides preferably comprise a sequence containing from about 8 to about 100 nucleotides and more preferably the FGF-X antisense oligonucleotides comprise from about 15 to about 30 nucleotides. The FGF-X antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linages (Uhlmann and Peyman, *Chemical Reviews 90*:543-548 1990; Schneider and Banner, *Tetrahedron Lett. 31*:335, 1990 which are incorporated by reference), modified nucleic acid bases and/or sugars and the like.

Another use of a variant FGF-X protein is as an FGF-X antagonist. A mutant or otherwise altered FGF-X molecule is constructed which binds to its receptor but does not activate the receptor. This protein is expected to compete with wild-type FGF-X for binding, and therefore inhibit the effects of FGF-X. Uses for such a protein include treatment of cancers, treatment of hyperproliferative conditions, and treatment of diseases that depend on activity of FGF-X.

The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example

intravenous, subcutaneous, intramuscular, transdermal, intrathecal or intracerebral. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation.

When it is intended that FGF-X be administered to cells of the pulmonary system, it can be administered in aerosol form, for example as an inhaler or as a nasal spray, for administration to reach tissues including the trachea, the bronchial tube, and alveolar cells. It can also be administered intravenously to reach pulmonary tissue and vessels.

FGF-X can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, FGF-X can be coupled to any substance known in the art to promote penetration or transport across the blood-brain barrier such as an antibody to the transferrin receptor, and administered by intravenous injection (See, for example, Friden et al., *Science 259*:373-377, 1993 which is incorporated by reference). Furthermore, FGF-X can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. (See, for example, Davis et al., *Enzyme Eng. 4*:169-73, 1978; Burnham, *Am. J. Hosp. Pharm. 51*:210-218, 1994 which are incorporated by reference).

The compositions are usually employed in the form of pharmaceutical preparations. Such preparations are made in a manner well known in the pharmaceutical art. One preferred preparation utilizes a vehicle of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous. FGF-X can also be incorporated into a solid or semi-solid biologically compatible matrix which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

It is also contemplated that certain formulations containing FGF-X are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Such

calculations can be made without undue experimentation by one skilled in the art in light of the activity disclosed herein in assay preparations of target cells. Exact dosages are determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration.

In one embodiment of this invention, FGF-X may be therapeutically administered by implanting into patients vectors or cells capable of producing a biologically-active form of FGF-X or a precursor of FGF-X, *i.e.*, a molecule that can be readily converted to a biological-active form of FGF-X by the body. In one approach cells that secrete FGF-X may be encapsulated into semipermeable membranes for implantation into a patient. The cells can be cells that normally express FGF-X or a precursor thereof or the cells can be transformed to express FGF-X or a precursor thereof. It is preferred that the cell be of human origin and that the FGF-X be human FGF-X when the patient is human. However, the formulations and methods herein can be used for veterinary as well as human applications and the term "patient" as used herein is intended to include human and veterinary patients.

Cells can be grown ex vivo for use in transplantation or engraftment into patients (Muench et al., *Leuk. & Lymph. 16*:1-11, 1994 which is incorporated by reference). In another embodiment of the present invention, FGF-X is used to promote the ex vivo expansion of a cells for transplantation or engraftment. Current methods have used bioreactor culture systems containing factors such as erythropoietin, colony stimulating factors, stem cell factor, and interleukins to expand hematopoietic progenitor cells for erythrocytes, monocytes, neutrophils, and lymphocytes (Verfaillie, *Stem Cells 12*:466-476, 1994 which is incorporated by reference). These stem cells can be isolated from the marrow of human donors, from human peripheral blood, or from umbilical cord blood cells. The expanded blood cells are used to treat patients who lack these cells as a result of specific disease conditions or as a result of high dose

chemotherapy for treatment of malignancy (George, *Stem Cells 12*(Suppl 1):249-255, 1994 which is incorporated by reference). In the case of cell transplant after chemotherapy, autologous transplants can be performed by removing bone marrow cells before chemotherapy, expanding the cells *ex vivo* using methods that also function to purge malignant cells, and transplanting the expanded cells back into the patient following chemotherapy (for review, see Rummel and Van Zant, *J. Hematotherapy 3*:213-218, 1994 which is incorporated by reference). Since FGF-X is expressed in the trachea, it is believed that FGF-X can function to regulate the proliferation or function of cells of the pulmonary system. Thus, the addition of FGF-X to culture systems used for ex vivo expansion of cells could stimulate the rate at which certain populations of cells multiply or differentiate, and improve the effectiveness of these expansion systems in generating cells needed for transplant.

In a number of circumstances it would be desirable to determine the levels of FGF-X in a patient. The identification of FGF-X along with the present report showing that FGF-X is expressed by trachea provides the basis for the conclusion that the presence of FGF-X serves a normal physiologic function related to cell growth and survival. Endogenously produced FGF-X may also play a role in certain disease conditions, particularly where there is cellular degeneration such as in conditions or diseases related to pulmonary dysfunction.

Given that FGF-X is expressed in trachea, it is likely that the level of FGF-X may be altered in a variety of conditions and that quantification of FGF-X levels would provide clinically useful information. Furthermore, in the treatment of degenerative conditions, compositions containing FGF-X can be administered and it would likely be desirable to achieve certain target levels of FGF-X in sera, in pulmonary fluid or in any desired tissue compartment. It would, therefore, be advantageous to be able to monitor the levels of FGF-X in a patient. Accordingly, the present invention also provides methods for detecting the presence of FGF-X in a sample from a patient.

The term "detection" as used herein in the context of detecting the presence of FGF-X in a patient is intended to include the determining of the amount of

FGF-X or the ability to express an amount of FGF-X in a patient, the distinguishing of FGF-X from other growth factors, the estimation of prognosis in terms of probable outcome of a degenerative disease and prospect for recovery, the monitoring of the FGF-X levels over a period of time as a measure of status of the condition, and the monitoring of FGF-X levels for determining a preferred therapeutic regimen for the patient.

To detect the presence of FGF-X in a patient, a sample is obtained from the patient. The sample can be a tissue biopsy sample or a sample of blood, plasma, serum, CSF, sputum, or the like. Samples for detecting FGF-X can be taken from any of tissues expressing FGF-X. When assessing peripheral levels of FGF-X, it is preferred that the sample be a sample of blood, plasma or serum. When assessing the levels of FGF-X in the pulmonary system a preferred sample is a sample obtained from the lungs.

In some instances it is desirable to determine whether the FGF-X gene is intact in the patient or in a tissue or cell line within the patient. By an intact FGF-X gene it is meant that there are no alterations in the gene such as point mutations, deletions, insertions, chromosomal breakage, chromosomal rearrangements and the like wherein such alteration might alter production of FGF-X or alter its biological activity, stability or the like to lead to disease processes or susceptibility to cellular degenerative conditions. Thus, in one embodiment of the present invention a method is provided for detecting and characterizing any alterations in the FGF-X gene. The method comprises providing an oligonucleotide that contains the FGF-X cDNA, genomic DNA or a fragment thereof or a derivative thereof. By a derivative of an oligonucleotide, it is meant that the derived oligonucleotide is substantially the same as the sequence from which it is derived in that the derived sequence has sufficient sequence complementarily to the sequence from which it is derived to hybridize to the FGF-X gene. The derived nucleotide sequence is not necessarily physically derived from the nucleotide sequence, but may be generated in any manner including for example, chemical synthesis or DNA replication or reverse transcription or transcription.

Typically, patient genomic DNA is isolated from a cell sample from the patient and digested with one or more restriction endonucleases such as, for example, TaqI and AluI. Using the Southern blot protocol, which is well known in the art, this assay determines whether a patient or a particular tissue in a patient has an intact FGF-X gene or an FGF-X gene abnormality.

Hybridization to an FGF-X gene would involve denaturing the chromosomal DNA to obtain a single-stranded DNA; contacting the single-stranded DNA with a gene probe associated with the FGF-X gene sequence; and identifying the hybridized DNA-probe to detect chromosomal DNA containing at least a portion of a human FGF-X gene.

The term "probe" as used herein refers to a structure comprised of a polynucleotide that forms a hybrid structure with a target sequence, due to complementarity of probe sequence with a sequence in the target region. Oligomers suitable for use as probes may contain a minimum of about 8-12 contiguous nucleotides which are complementary to the targeted sequence and preferably a minimum of about 20.

The FGF-X gene probes of the present invention can be DNA or RNA oligonucleotides and can be made by any method known in the art such as, for example, excision, transcription or chemical synthesis. Probes may be labeled with any detectable label known in the art such as, for example, radioactive or fluorescent labels or enzymatic marker. Labeling of the probe can be accomplished by any method known in the art such as by PCR, random priming, end labeling, nick translation or the like. One skilled in the art will also recognize that other methods not employing a labeled probe can be used to determine the hybridization. Examples of methods that can be used for detecting hybridization include Southern blotting, fluorescence in situ hybridization, and single-strand conformation polymorphism with PCR amplification.

Hybridization is typically carried out at 25° - 45° C, more preferably at 32° - 40° C and more preferably at 37° - 38° C. The time required for hybridization is from about 0.25 to about 96 hours, more preferably from about one to about 72 hours, and most preferably from about 4 to about 24 hours.

FGF-X gene abnormalities can also be detected by using the PCR method and primers that flank or lie within the FGF-X gene. The PCR method is well known in the art. Briefly, this method is performed using two oligonucleotide primers which are capable of hybridizing to the nucleic acid sequences flanking a target sequence that lies within an FGF-X gene and amplifying the target sequence. The terms "oligonucleotide primer" as used herein refers to a short strand of DNA or RNA ranging in length from about 8 to about 30 bases. The upstream and downstream primers are typically from about 20 to about 30 base pairs in length and hybridize to the flanking regions for replication of the nucleotide sequence. The polymerization is catalyzed by a DNA-polymerase in the presence of deoxynucleotide triphosphates or nucleotide analogs to produce double-stranded DNA molecules. The double strands are then separated by any denaturing method including physical, chemical or enzymatic. Commonly, the method of physical denaturation is used involving heating the nucleic acid, typically to temperatures from about 80°C. to 105°C. for times ranging from about 1 to about 10 minutes. The process is repeated for the desired number of cycles.

The primers are selected to be substantially complementary to the strand of DNA being amplified. Therefore, the primers need not reflect the exact sequence of the template, but must be sufficiently complementary to selectively hybridize with the strand being amplified.

After PCR amplification, the DNA sequence comprising FGF-X or pre-pro FGF-X or a fragment thereof is then directly sequenced and analyzed by comparison of the sequence with the sequences disclosed herein to identify alterations which might change activity or expression levels or the like.

In another embodiment, a method for detecting FGF-X is provided based upon an analysis of tissue expressing the FGF-X gene. Certain tissues such as those identified below in Example 1 have been found to express the FGF-X gene. The method comprises hybridizing a polynucleotide to mRNA from a sample of tissues that normally express the FGF-X gene. The sample is obtained from a patient suspected of having an abnormality in the FGF-X gene or in the FGF-X gene of particular cells. The polynucleotide comprises SEQ ID NO:1 or a derivative thereof or a fragment thereof.

To detect the presence of mRNA encoding FGF-X protein, a sample is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis or other size separation techniques.

The mRNA of the sample is contacted with a DNA sequence serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

When using the cDNA encoding FGF-X protein or a derivative of the cDNA as a probe, high stringency conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of FGF-X nucleotide sequences when in fact an intact and functioning FGF-X gene is not present. When using sequences derived from the FGF-X cDNA, less stringent conditions could be used, however, this would be a less preferred approach because of the likelihood of false positives. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook et al. (Sambrook, et al., 1989, supra).

In order to increase the sensitivity of the detection in a sample of mRNA encoding the FGF-X protein, the technique of reverse transcription/polymerization chain reaction (RT/PCR) can be used to amplify cDNA transcribed from mRNA encoding the FGF-X protein. The method of RT/PCR is well known in the art.

The RT/PCR method can be performed as follows. Total cellular RNA is isolated by, for example, the standard guanidium isothiocyanate method and the total RNA is reverse transcribed. The reverse transcription method involves synthesis of DNA on a template of RNA using a reverse transcriptase enzyme and a 3' end primer. Typically, the primer contains an oligo(dT) sequence. The cDNA thus produced is then amplified using the PCR method and FGF-X specific primers. (Belyavsky et al., *Nucl. Acid Res. 17*:2919-2932, 1989; Krug and Berger, *Methods in Enzymology*, *152*:316-325, Academic Press, NY, 1987 which are incorporated by reference).

The polymerase chain reaction method is performed as described above using two oligonucleotide primers that are substantially complementary to the two flanking regions of the DNA segment to be amplified.

Following amplification, the PCR product is then electrophoresed and detected by ethidium bromide staining or by phosphoimaging.

The present invention further provides for methods to detect the presence of the FGF-X protein in a sample obtained from a patient. Any method known in the art for detecting proteins can be used. Such methods include, but are not limited to immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical techniques, agglutination and complement assays. (for example, see *Basic and Clinical Immunology*, 217-262, Sites and Terr, eds., Appleton & Lange, Norwalk, CT, 1991 which is incorporated by reference). Preferred are binder-ligand immunoassay methods including reacting antibodies with an epitope or epitopes of the FGF-X protein and competitively displacing a labeled FGF-X protein or derivative thereof. Preferred antibodies are prepared according to Example 2.

As used herein, a derivative of the FGF-X protein is intended to include a polypeptide in which certain amino acids have been deleted or replaced or changed to modified or unusual amino acids wherein the FGF-X derivative is biologically equivalent to FGF-X and wherein the polypeptide derivative cross-reacts with antibodies raised against the FGF-X protein. By cross-reaction it is meant that an antibody reacts with an antigen other than the one that induced its formation.

Numerous competitive and non-competitive protein binding immunoassays are well known in the art. Antibodies employed in such assays may be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA), enzyme immunoassays, *e.g.*, enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like.

Polyclonal or monoclonal antibodies to the FGF-X protein or an epitope thereof can be made for use in immunoassays by any of a number of methods known in

the art. By epitope reference is made to an antigenic determinant of a polypeptide. An epitope could comprise 3 amino acids in a spacial conformation which is unique to the epitope. Generally an epitope consists of at least 5 such amino acids. Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and 2 dimensional nuclear magnetic resonance.

One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesizing the sequence and injecting it into an appropriate animal, usually a rabbit or a mouse (see Example 2).

Oligopeptides can be selected as candidates for the production of an antibody to the FGF-X protein based upon the oligopeptides lying in hydrophilic regions, which are thus likely to be exposed in the mature protein. Oligopeptides for raising antibodies include the contiguous amino acids between Glu 56 and Arg 71, or Ser 75 and Gly 89, of SEQ ID NO:2.

Antibodies to FGF-X can also be raised against oligopeptides that include one or more of the conserved regions of two or more FGF molecules such that the antibody can cross-react with other family members. Such antibodies can be used to identify and isolate other family members.

Methods for preparation of the FGF-X protein or an epitope thereof include, but are not limited to chemical synthesis, recombinant DNA techniques or isolation from biological samples. Chemical synthesis of a peptide can be performed, for example, by the classical Merrifeld method of solid phase peptide synthesis (Merrifeld, *J. Am. Chem. Soc. 85*:2149, 1963 which is incorporated by reference) or the FMOC strategy on a Rapid Automated Multiple Peptide Synthesis system (E. I. du Pont de Nemours Company, Wilmington, DE) (Caprino and Han, *J Org Chem 37*:3404, 1972 which is incorporated by reference).

Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by subsequent boosts at appropriate intervals. The animals are bled and sera assayed against purified FGF-X protein usually by ELISA or by bioassay based upon the ability to block the action of FGF-X on neurons

or other cells. When using avian species, *e.g.*, chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared after the method of Milstein and Kohler by fusing splenocytes from immunized mice with continuously replicating tumor cells such as myeloma or lymphoma cells. (Milstein and Kohler, *Nature 256*:495-497, 1975; Gulfre and Milstein, *Methods in Enzymology: Immunochemical Techniques 73*:1-46, Langone and Banatis eds., Academic Press, 1981 which are incorporated by reference). The hybridoma cells so formed are then cloned by limiting dilution methods and supernates assayed for antibody production by ELISA, RIA or bioassay.

The unique ability of antibodies to recognize and specifically bind to target proteins provides an approach for treating an over-expression of the protein. Thus, another aspect of the present invention provides for a method for preventing or treating diseases involving over-expression of the FGF-X protein by treatment of a patient with specific antibodies to the FGF-X protein.

Specific antibodies, either polyclonal or monoclonal, to the FGF-X protein can be produced by any suitable method known in the art as discussed above. For example, murine or human monoclonal antibodies can be produced by hybridoma technology or, alternatively, the FGF-X protein, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to the FGF-X protein. Such antibodies can be from any class of antibodies including, but not limited to IgG, IgA, IgM, IgD, and IgE or in the case of avian species, IgY and from any subclass of antibodies.

Polypeptides encoded by the instant polynucleotides and corresponding full-length genes can be used to screen peptide libraries, protein libraries, small molecule libraries, and phage display libraries, and other known methods, to identify analogs or antagonists.

Native FGF polypeptides may play a role in cancer. For example, FGF family members can induce marked morphological transformation of NIH 3T3 cells, and exhibit strong tumorigenicity in nude mice. Angiogenic activity has been exhibited

by FGF family members. Thus, inhibitors of FGF can be used to treat cancer, such as prostate.

A library of peptides may be synthesized following the methods disclosed in U.S. Patent No. 5,010,175, and in PCT No. WO 91/17823. As described below in brief, a mixture of peptides is prepared, which is then screened to identify the peptides exhibiting the desired signal transduction and receptor binding activity. According to the method of the '175 patent, a suitable peptide synthesis support (*e.g.*, a resin) is coupled to a mixture of appropriately protected, activated amino acids. The concentration of each amino acid in the reaction mixture is balanced or adjusted in inverse proportion to its coupling reaction rate so that the product is an equimolar mixture of amino acids coupled to the starting resin. The bound amino acids are then deprotected, and reacted with another balanced amino acid mixture to form an equimolar mixture of all possible dipeptides. This process is repeated until a mixture of peptides of the desired length (*e.g.*, hexamers) is formed. Note that one need not include all amino acids in each step: one may include only one or two amino acids in some steps (*e.g.*, where it is known that a particular amino acid is essential in a given position), thus reducing the complexity of the mixture. After the synthesis of the peptide library is completed, the mixture of peptides is screened for binding to the selected polypeptide. The peptides are then tested for their ability to inhibit or enhance activity. Peptides exhibiting the desired activity are then isolated and sequenced.

The method described in PCT No. WO 91/17823 is similar. However, instead of reacting the synthesis resin with a mixture of activated amino acids, the resin is divided into twenty equal portions (or into a number of portions corresponding to the number of different amino acids to be added in that step), and each amino acid is coupled individually to its portion of resin. The resin portions are then combined, mixed, and again divided into a number of equal portions for reaction with the second amino acid. In this manner, each reaction may be easily driven to completion. Additionally, one may maintain separate "subpools" by treating portions in parallel, rather than combining all resins at each step. This simplifies the process of determining

which peptides are responsible for any observed receptor binding or signal transduction activity.

In such cases, the subpools containing, *e.g.*, 1-2,000 candidates each are exposed to one or more polypeptides of the invention. Each subpool that produces a positive result is then resynthesized as a group of smaller subpools (sub-subpools) containing, *e.g.*, 20-100 candidates, and reassayed. Positive sub-subpools may be resynthesized as individual compounds, and assayed finally to determine the peptides that exhibit a high binding constant. These peptides can be tested for their ability to inhibit or enhance the native activity. The methods described in PCT No. WO 91/7823 and U.S. Patent No. 5,194,392 (herein incorporated by reference) enable the preparation of such pools and subpools by automated techniques in parallel, such that all synthesis and resynthesis may be performed in a matter of days.

Peptide agonists or antagonists are screened using any available method, such as signal transduction, antibody binding, receptor binding and mitogenic assays. The assay conditions ideally should resemble the conditions under which the native activity is exhibited *in vivo*, that is, under physiologic pH, temperature, and ionic strength. Suitable agonists or antagonists will exhibit strong inhibition or enhancement of the native activity at concentrations that do not cause toxic side effects in the subject. Agonists or antagonists that compete for binding to the native polypeptide may require concentrations equal to or greater than the native concentration, while inhibitors capable of binding irreversibly to the polypeptide may be added in concentrations on the order of the native concentration.

The therapeutic FGF-X polynucleotides and polypeptides of the present invention may be utilized in gene delivery vehicles. The gene delivery vehicle may be of viral or non-viral origin (*see generally*, Jolly, *Cancer Gene Therapy 1*:51-64 (1994); Kimura, *Human Gene Therapy 5*:845-852 (1994); Connelly, *Human Gene Therapy 1*:185-193 (1995); and Kaplitt, *Nature Genetics 6*:148-153 (1994)). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can

be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

The present invention can employ recombinant retroviruses which are constructed to carry or express a selected nucleic acid molecule of interest. Retrovirus vectors that can be employed include those described in EP 0 415 731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218; Vile and Hart, *Cancer Res. 53*:3860-3864 (1993); Vile and Hart, *Cancer Res. 53*:962-967 (1993); Ram et al., *Cancer Res. 53*:83-88 (1993); Takamiya et al., *J. Neurosci. Res. 33*:493-503 (1992); Baba et al., *J. Neurosurg. 79*:729-735 (1993); U.S. Patent No. 4,777,127; GB Patent No. 2,200,651; and EP 0 345 242. Preferred recombinant retroviruses include those described in WO 91/02805.

Packaging cell lines suitable for use with the above-described retroviral vector constructs may be readily prepared (see PCT publications WO 95/30763 and WO 92/05266), and used to create producer cell lines (also termed vector cell lines) for the production of recombinant vector particles. Within particularly preferred embodiments of the invention, packaging cell lines are made from human (such as HT1080 cells) or mink parent cell lines, thereby allowing production of recombinant retroviruses that can survive inactivation in human serum.

The present invention also employs alphavirus-based vectors that can function as gene delivery vehicles. Such vectors can be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532). Representative examples of such vector systems include those described in U.S. Patent Nos. 5,091,309; 5,217,879; and 5,185,440; and PCT Publication Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994.

Gene delivery vehicles of the present invention can also employ parvovirus such as adeno-associated virus (AAV) vectors. Representative examples include the AAV vectors disclosed by Srivastava in WO 93/09239, Samulski et al., *J.*

Vir. 63:3822-3828 (1989); Mendelson et al., *Virol. 166*:154-165 (1988); and Flotte et al., *P.N.A.S. 90*:10613-10617 (1993).

Representative examples of adenoviral vectors include those described by Berkner, *Biotechniques 6*:616-627 (Biotechniques); Rosenfeld et al., *Science 252*:431-434 (1991); WO 93/19191; Kolls et al., *P.N.A.S.* :215-219 (1994); Kass-Eisler et al., *P.N.A.S. 90*:11498-11502 (1993); Guzman et al., *Circulation 88*:2838-2848 (1993); Guzman et al., *Cir. Res. 73*:1202-1207 (1993); Zabner et al., *Cell 75*:207-216 (1993); Li et al., *Hum. Gene Ther. 4*:403-409 (1993); Cailaud et al., *Eur. J. Neurosci. 5*:1287-1291 (1993); Vincent et al., *Nat. Genet. 5*:130-134 (1993); Jaffe et al., *Nat. Genet. 1*:372-378 (1992); and Levrero et al., *Gene 101*:195-202 (1992). Exemplary adenoviral gene therapy vectors employable in this invention also include those described in WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655. Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther. 3*:147-154 (1992) may be employed.

Other gene delivery vehicles and methods may be employed, including polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example Curiel, *Hum. Gene Ther. 3*:147-154 (1992); ligand-linked DNA, for example see Wu, *J. Biol. Chem. 264*:16985-16987 (1989); eukaryotic cell delivery vehicles cells, for example see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial No. 08/404,796; deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; ionizing radiation as described in U.S. Patent No. 5,206,152 and in WO 92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip, *Mol. Cell Biol. 14*:2411-2418 (1994), and in Woffendin, *Proc. Natl. Acad. Sci. 91*:1581-1585 (1994).

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity

and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Patent Publication Nos. WO 95/13796, WO 94/23697, and WO 91/14445, and EP No. 0 524 968.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al., *Proc. Natl. Acad. Sci. USA* *91*(24):11581-11585 (1994). Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Patent No. 5,206,152 and PCT Patent Publication No. WO 92/11033.

FGF has been implicated in diseases characterized by loss of function, inadequate function/number, abnormal function or death of cells, tissues or organs for which function or survival can be prolonged/rescued, and abnormalities reversed or prevented by therapy with FGF.

Such diseases can include loss of pulmonary, bronchial or alveolar cells or function, healing of pulmonary or bronchial wounds, pulmonary infraction, emphysema/chronic obstructive pulmonary disease, asthma, sequelae of infectious or autoimmune disease, sequelae of pulmonary arterial or venous hypertension, pulmonary fibrosis, pulmonary disease of immaturity, and cystic fibrosis.

Other such diseases can include thyroid diseases including thyroid tumors and cancer, autoimmune and inflammatory thyroid disease, hypothyroidism and hyperthyroidism/Grave's disease. Additional, diseases include parathyroid diseases including parathyroid tumors and cancer, hyperparathyroidism and hypoparathyroidism. Also included are diseases of the eye including macular degeneration, retinitis pigmentosa, retinal detachment, ischemic retinal disease, and the like.

Ischemic vascular disease may be amenable to FGF-X treatment, wherein the disease is characterized by inadequate blood flow to an organ(s).

Treatment may induce therapeutic angiogenesis or preserve function/survival of cells (myocardial ischemia/infarction, peripheral vascular disease, renal artery disease, stroke). Cardiomyopathies characterized by loss of function or death of cardiac myocytes or supporting cells in the heart (congestive heart failure, myocarditis) may also be treated using FGF-X, as can musculoskeletal disease characterized by loss of function, inadequate function or death of skeletal muscle cells, bone cells or supporting cells. Examples include skeletal myopathies, bone disease, and arthritis.

FGF-X polynucleotides and polypeptides may aid in correction of congenital defects due to loss of FGF-X molecule or its function in, for example, heart, lung, brain, limbs, and kidney.

Treatment of wound healing is yet another use of FGF-X polypeptides and polynucleotides, either due to trauma, disease, medical or surgical treatment, including regeneration of cell populations and tissues depleted by these processes. Examples include liver regeneration, operative wound healing, re-endothelialization of injured blood vessels, healing of traumatic wounds, healing of ulcers due to vascular, metabolic disease, bone fractures and loss of cells due to inflammatory disease.

FGF-X may also be used in screens to identify drugs for treatment of cancers which involve over-activity of the molecule, or new targets which would be useful in the identification of new drugs.

For all of the preceding embodiments, the clinician will determine, based on the specific condition, whether FGF-X polypeptides or polynucleotides, antibodies to FGF-X, or small molecules such as peptide analogues or antagonists, will be the most suitable form of treatment. These forms are all within the scope of the invention.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

EXAMPLES

EXAMPLE 1

FGF-X EXPRESSION PATTERNS

cDNA libraries were analyzed to determine expression of FGF-X, as described in detail below. By Northern blot, high levels of expression were seen in trachea. Low to no expression was seen in stomach, thyroid, spinal cord, lymph node, adrenal gland, bone marrow, peripheral blood lymphocytes, spleen, thymus, prostate, testes, uterus, small intestine, colon, ovary, and fetal liver (Figure 1).

Commercially prepared RNA blots of human poly A RNA from various tissues were prehybridized with hybridization reagent from BIOS at 65°C for four hours. The 500 bp PCR product of val162-val166 reaction was gel purified and radioactively labelled with ^{32}P deoxy nucleotides using the reagents from random primer kit by Boehringer. The probe was purified on a Sephadex-G50 column and the blots were hybridized overnight with the probe in 10 ml of hybridization mix containing 10^6 cpm/ml. The blots were sequentially washed twice with 2X SSC, 0.1% SDS at room temperature and with 1X SSC. 0.1% SDS at 55°C and exposed to Kodak film with amplifying screens at -70°C overnight.

EXAMPLE 2

PREPARATION OF ANTISERA TO FGF-X BY IMMUNIZATION OF RABBITS WITH AN FGF-X PEPTIDE.

A peptide sequence corresponding to selected contiguous amino acids of the human FGF-X protein is synthesized and coupled to keyhole limpet hemocyanin (KLH) as described (Harlow and Land, Antibodies: A Laboratory Manual, 1988. Cold Spring Harbor Laboratory, New York, NY). The KLH-coupled peptide is used to immunize rabbits. Antisera are tested for specificity to FGF-X, and for cross-reactivity with other FGF proteins.

Two exemplary peptide sequences are:

1. EIRPDGYNVRSEKHR (amino acids 56-71 of SEQ ID NO:2)
2. SLSSAKQRQLYKNRG (amino acids 75-89 of SEQ ID NO:2)

EXAMPLE 3

FGF-X EXPRESSION IN HUMAN TISSUES

Primer val164 (TTCTTGTAGAAGCACGTC-nt539-nt521, antisense) was used to reverse transcribe 2 μg Poly A^+ RNA from human brain, lung and thyroid with the enzyme Superscript II reverse transcriptase at 50°C for 30 min in a final volume of 20 μls according to the manufacturer's protocol. Following RNase treatment of the reverse transcription mix, 1 μl of the aliquot was used for first round of PCR amplification with primers val161 (GGTACCACAGCCCCTGGCAGCAGT-nt509-486, antisense) and val165 (TACACGTACAGTGTGTACATCA-, sense) in a final volume of 50 μls. The amplification conditions were heat denaturation at 94°C for 2 min, followed by 40 cycles of 30 sec at 94°C, 30 sec at 50°C, 1 min at 68°C. A one μl aliquot of the first round PCR amplified material was used for second round amplification with PCR primers val162 (GAAGAGGCCCGGGCATGGTCTCAG-nt485-462, antisense) and val1166 (GGAAGTATGTATAAAACATGCAA-nt1-23, sense). The amplification conditions were as described above. 5 μl aliquot of the second round PCR amplified material was electrophoresed on 1% agarose gel and a product of 500bp (arrow) was visualized by ethidium bromide staining. Size markers are shown at left (Figure 3).

All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

Although certain preferred embodiments have been described herein, it is not intended that such embodiments be construed as limitations on the scope of the invention except as set forth in the following claims.

CLAIMS

WE CLAIM:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding amino acids from about 1 to about 147 of SEQ ID NO:2;

(b) a polynucleotide encoding amino acids from about 2 to about 147 of SEQ ID NO:2;

(c) a polynucleotide encoding amino acids from about 56 to about 71 of SEQ ID NO:2;

(d) a polynucleotide encoding amino acids from about 75 to about 89 of SEQ ID NO:2;

(e) the polynucleotide complement of (a), (b), (c), or (d); and

(f) a polynucleotide at least 90% identical to the polynucleotide of (a), (b), (c), or (d).

2. An isolated nucleic acid molecule which comprises 20-420 contiguous nucleotides from the coding region of SEQ ID NO:1.

3. The isolated nucleic acid molecule of claim 2, which comprises 60-400 contiguous nucleotides from the coding region of SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 3, which comprises 200-300 contiguous nucleotides from the coding region of SEQ ID NO:1.

5. An isolated nucleic acid molecule comprising a polynucleotide encoding a polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) amino acids from about 1 to about 147 of SEQ ID NO:2;

(b) amino acids from about 2 to about 147 of SEQ ID NO:2;

(c) amino acids from about 56 to about 71 of SEQ ID NO:2; and

(d) amino acids from about 75 to about 89 of SEQ ID NO:2.

6. The isolated nucleic acid molecule of claim 1, which is DNA.

7. A method of making a recombinant vector comprising inserting a nucleic acid molecule of claim 1 into a vector in operable linkage to a promoter.

8. A recombinant vector produced by the method of claim 7.

9. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 8 into a host cell.

10. A recombinant host cell produced by the method of claim 9.

11. A recombinant method of producing a polypeptide, comprising culturing the recombinant host cell of claim 10 under conditions such that said polypeptide is expressed and recovering said polypeptide.

12. An isolated polypeptide comprising amino acids at least 95% identical to amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 147 of SEQ ID NO:2;

(b) amino acids from about 2 to about 147 of SEQ ID NO:2;

(c) amino acids from about 56 to about 71 of SEQ ID NO:2; and

(d) amino acids from about 75 to about 89 of SEQ ID NO:2.

13. An isolated polypeptide wherein, except for at least one conservative amino acid substitution, said polypeptide has an amino acid sequence selected from the group consisting of:

(a) amino acids from about 1 to about 147 of SEQ ID NO:2;

(b) amino acids from about 2 to about 147 of SEQ ID NO:2;

(c) amino acids from about 56 to about 71 of SEQ ID NO:2; and

(d) amino acids from about 75 to about 89 of SEQ ID NO:2.

14. An isolated polypeptide comprising amino acids selected from the group consisting of:

(a) amino acids from about 1 to about 147 of SEQ ID NO:2;

(b) amino acids from about 2 to about 147 of SEQ ID NO:2;

(c) amino acids from about 56 to about 71 of SEQ ID NO:2; and

(d) amino acids from about 75 to about 89 of SEQ ID NO:2.

15. An epitope-bearing portion of the polypeptide of SEQ ID NO:2.

16. The epitope-bearing portion of claim 15, which comprises between 10 and 50 contiguous amino acids of SEQ ID NO:2.

17. The epitope-bearing portion of claim 15, which comprises amino acids EIRPDGYNVRSEKHR (SEQ ID NO:3).

18. The epitope-bearing portion of claim 15, which comprises amino acids SLSSAKORQLYKNRG (SEQ ID NO:4).

19. An isolated antibody that binds specifically to the polypeptide of claim 12.

20. An isolated antibody that binds specifically to the polypeptide of claim 13.

21. An isolated antibody that binds specifically to the polypeptide of claim 14.

22. A pharmaceutical composition comprising the polypeptide of claim 12, in combination with a pharmaceutically acceptable carrier.

23. A method for providing trophic support for cells in a patient in need thereof, the method comprising administering to the patient a composition comprising a polynucleotide encoding the polypeptide of SEQ ID NO:2.

24. The method of claim 20 wherein said polynucleotide is administered by implanting cells which express said polynucleotide into the patient, wherein said cells express FGF-X polypeptide in the patient.

25. The method of claim 23 wherein the implanted cells are encapsulated in a semipermeable membrane.

26. The method of claim 23 wherein the patient suffers from a condition selected from the group consisting of pulmonary infarction, emphysema, chronic obstructive pulmonary disease, asthma, infection, autoimmune disease, pulmonary arterial hypertension, pulmonary venous hypertension, pulmonary fibrosis, pulmonary disease of immaturity, cystic fibrosis, pulmonary injury, bronchial injury, or tracheal injury.

27. The method of claim 26 wherein the condition is cystic fibrosis.

28. The method of claim 26 wherein the condition is chronic obstructive pulmonary disease.

29. The method of claim 26 wherein the condition is asthma.

30. A kit for detecting the presence of mRNA encoding FGF-X in a sample from a patient, said kit comprising a polynucleotide having at least 20 contiguous nucleotides of the polynucleotide of claim 1, packaged in a container.

31. The kit according to claim 19 wherein the polynucleotide encodes SEQ ID NO:2.

32. A kit for detecting the presence of FGF-X polypeptide in a sample from a patient, said kit comprising an antibody according to claim 9, packaged in a container.

33. The method of claim 23 wherein the patient suffers from a condition characterized by neuronal cell degeneration.

34. The method of claim 33 wherein the condition is Parkinson's disease.

35. A method for providing trophic support for cells in a patient in need thereof, the method comprising administering to the patient a composition comprising a polypeptide of SEQ ID NO:2.

36. The method of claim 35 wherein the patient suffers from a condition characterized by neuronal cell degeneration.

37. The method of claim 36 wherein the condition is Parkinson's

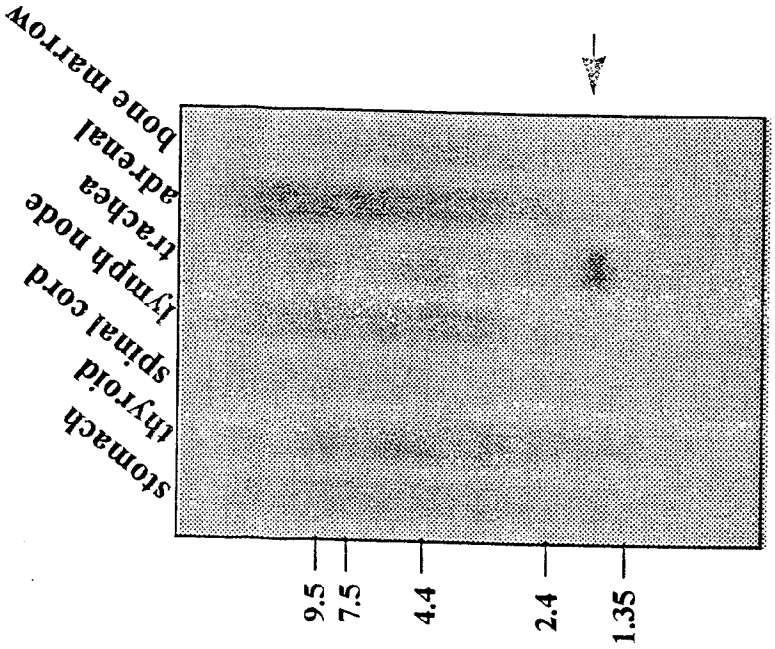
disease.

38. The method of claim 36 wherein the condition affects the substantia nigra.

39. A method of alleviating a disease condition in the brain of a human patient wherein said disease condition is alleviated by at least one method selected from the group consisting of slowing degeneration of, restoring function of, and increasing the number of, dopaminergic neurons in said human patient, said method comprising administering to said patient a pharmaceutically effective composition comprising a polypeptide having the amino acid sequence of SEQ ID NO:2.

40. A method of alleviating a disease condition in the brain of a human patient wherein said disease condition is alleviated by at least one method selected from the group consisting of slowing degeneration of, restoring function of, and increasing the number of, dopaminergic neurons in said human patient, said method comprising administering to said patient a pharmaceutically effective composition comprising a polynucleotide encoding the amino acid sequence of SEQ ID NO:2.

41. The method of claim 40 wherein said polynucleotide has the sequence of SEQ ID NO:1.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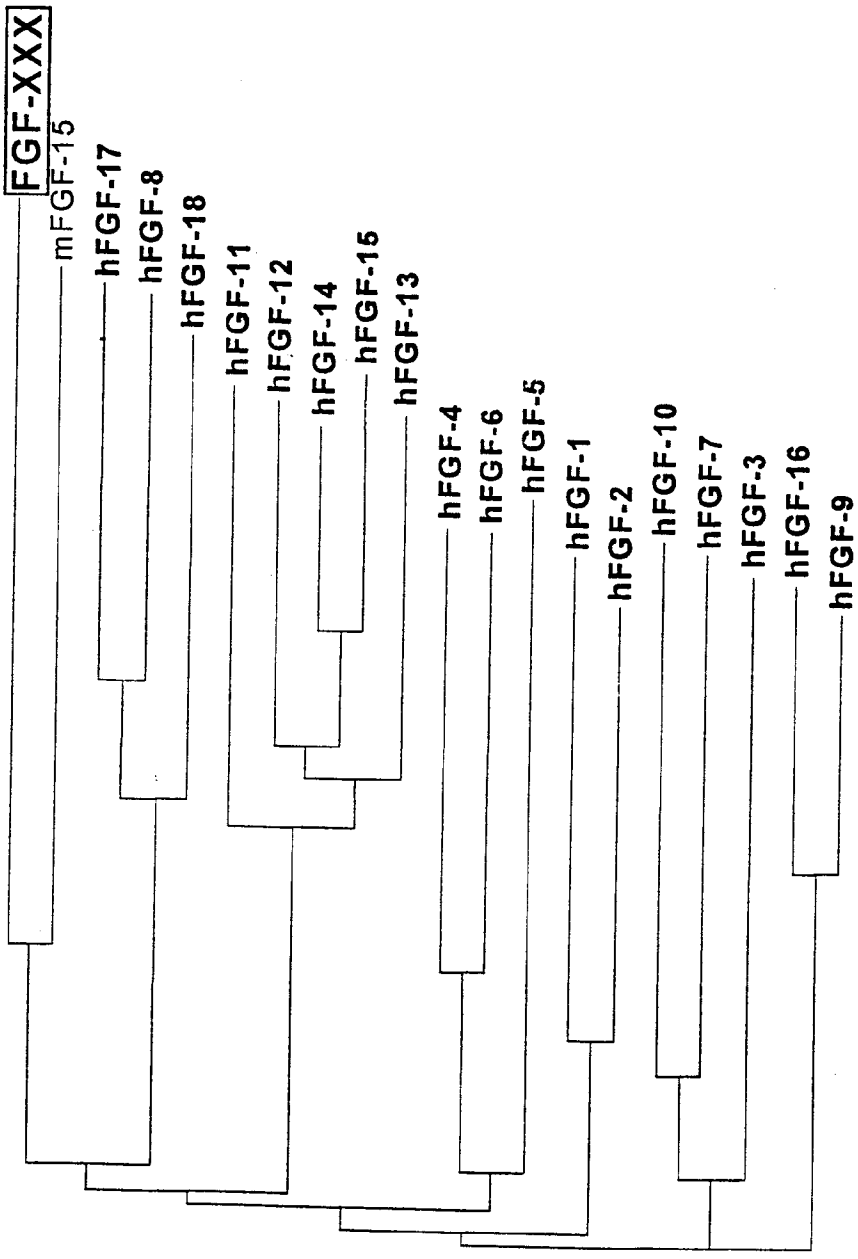
2.4
1.35

FIGURE 2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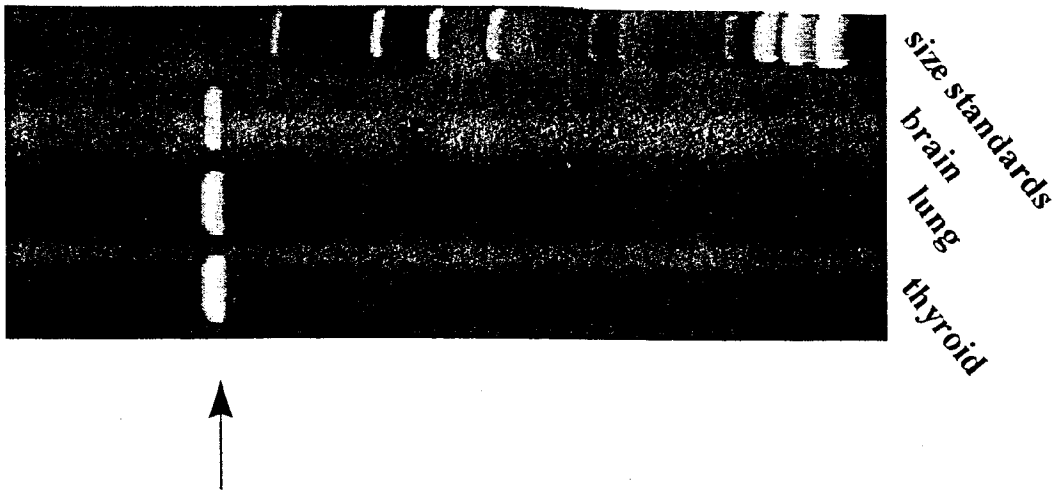

FIGURE 3

Figure 4

Codon usage for yeast (highly expressed) genes

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	33.00	0.86	0.01
Gly	GGA	70.00	1.82	0.02
Gly	GGT	2672.00	69.62	0.91
Gly	GGC	171.00	4.46	0.06
Glu	GAG	277.00	7.22	0.10
Glu	GAA	2442.00	63.63	0.90
Asp	GAT	1100.00	28.66	0.48
Asp	GAC	1211.00	31.55	0.52
Val	GTG	117.00	3.05	0.04
Val	GTA	75.00	1.95	0.03
Val	GTT	1548.00	40.33	0.56
Val	GTC	1026.00	26.73	0.37
Ala	GCG	36.00	0.94	0.01
Ala	GCA	203.00	5.29	0.06
Ala	GCT	2221.00	57.87	0.65
Ala	GCC	969.00	25.25	0.28
Arg	AGG	20.00	0.52	0.01
Arg	AGA	1336.00	34.81	0.83
Ser	AGT	116.00	3.02	0.05
Ser	AGC	94.00	2.45	0.04
Lys	AAG	2365.00	61.62	0.78
Lys	AAA	651.00	16.96	0.22
Asn	AAT	347.00	9.04	0.22
Asn	AAC	1259.00	32.80	0.78
Met	ATG	766.00	19.96	1.00
Ile	ATA	43.00	1.12	0.02
Ile	ATT	1223.00	31.87	0.52
Ile	ATC	1070.00	27.88	0.46
Thr	ACG	28.00	0.73	0.01

FIGURE 4 CONT'D

Thr	ACA	126.00	3.28	0.06
Thr	ACT	1129.00	29.42	0.50
Thr	ACC	962.00	25.07	0.43
Trp	TGG	325.00	8.47	1.00
End	TGA	10.00	0.26	0.09
Cys	TGT	254.00	6.62	0.89
Cys	TGC	33.00	0.86	0.11
End	TAG	11.00	0.29	0.10
End	TAA	85.00	2.21	0.80
Tyr	TAT	219.00	5.71	0.19
Tyr	TAC	913.00	23.79	0.81
Leu	TTG	2202.00	57.38	0.69
Leu	TTA	576.00	15.01	0.18
Phe	TTT	432.00	11.26	0.27
Phe	TTC	1145.00	29.83	0.73
Ser	TCG	26.00	0.68	0.01
Ser	TCA	149.00	3.88	0.06
Ser	TCT	1279.00	33.33	0.52
Ser	TCC	818.00	21.31	0.33
Arg	CGG	0.00	0.00	0.00
Arg	CGA	1.00	0.03	0.00
Arg	CGT	249.00	6.49	0.15
Arg	CGC	5.00	0.13	0.00
Gln	CAG	62.00	1.62	0.05
Gln	CAA	1225.00	31.92	0.95
His	CAT	236.00	6.15	0.35
His	CAC	433.00	11.28	0.65
Leu	CTG	52.00	1.35	0.02
Leu	CTA	236.00	6.15	0.07
Leu	CTT	90.00	2.35	0.03
Leu	CTC	14.00	0.36	0.00
Pro	CCG	10.00	0.26	0.01
Pro	CCA	1271.00	33.12	0.80
Pro	CCT	279.00	7.27	0.18
Pro	CCC	33.00	0.86	0.02

Figure 5

Codon usage for Drosophila (highly expressed) genes

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	6.00	0.28	0.00
Gly	GGA	380.00	18.04	0.22
Gly	GGT	575.00	27.29	0.34
Gly	GGC	746.00	35.41	0.44
Glu	GAG	1217.00	57.77	0.91
Glu	GAA	115.00	5.46	0.09
Asp	GAT	503.00	23.88	0.43
Asp	GAC	654.00	31.04	0.57
Val	GTG	719.00	34.13	0.45
Val	GTA	29.00	1.38	0.02
Val	GTT	226.00	10.73	0.14
Val	GTC	608.00	28.86	0.38
Ala	GCG	94.00	4.46	0.05
Ala	GCA	80.00	3.80	0.04
Ala	GCT	446.00	21.17	0.24
Ala	GCC	1277.00	60.61	0.67
Arg	AGG	48.00	2.28	0.06
Arg	AGA	12.00	0.57	0.01
Ser	AGT	16.00	0.76	0.01
Ser	AGC	267.00	12.67	0.23
Lys	AAG	1360.00	64.55	0.93
Lys	AAA	108.00	5.13	0.07
Asn	AAT	127.00	6.03	0.13
Asn	AAC	878.00	41.67	0.87
Met	ATG	387.00	18.37	1.00
Ile	ATA	4.00	0.19	0.00
Ile	ATT	390.00	18.51	0.29
Ile	ATC	969.00	45.99	0.71

FIGURE 5 CONT'D

Thr	ACG	114.00	5.41	0.08
Thr	ACA	34.00	1.61	0.02
Thr	ACT	164.00	7.78	0.11
Thr	ACC	1127.00	53.49	0.78
Trp	TGG	243.00	11.53	1.00
End	TGA	1.00	0.05	0.01
Cys	TGT	20.00	0.95	0.08
Cys	TGC	220.00	10.44	0.92
End	TAG	12.00	0.57	0.17
End	TAA	58.00	2.75	0.82
Tyr	TAT	113.00	5.36	0.16
Tyr	TAC	574.00	27.25	0.84
Leu	TTG	210.00	9.97	0.12
Leu	TTA	9.00	0.43	0.01
Phe	TTT	62.00	2.94	0.09
Phe	TTC	635.00	30.14	0.91
Ser	TCG	195.00	9.26	0.17
Ser	TCA	29.00	1.38	0.02
Ser	TCT	103.00	4.89	0.09
Ser	TCC	558.00	26.49	0.48
Arg	CGG	7.00	0.33	0.01
Arg	CGA	25.00	1.19	0.03
Arg	CGT	281.00	13.34	0.34
Arg	CGC	465.00	22.07	0.55
Gln	CAG	703.00	33.37	0.91
Gln	CAA	66.00	3.13	0.09
His	CAT	88.00	4.18	0.22
His	CAC	312.00	14.81	0.78
Leu	CTG	1182.00	56.10	0.69
Leu	CTA	21.00	1.00	0.01
Leu	CTT	55.00	2.61	0.03
Leu	CTC	224.00	10.63	0.13
Pro	CCG	84.00	3.99	0.09
Pro	CCA	135.00	6.41	0.15
Pro	CCT	72.00	3.42	0.08
Pro	CCC	626.00	29.71	0.68

Figure 6

Codon usage for enteric bacterial (highly expressed) genes 7/19/83

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	13.00	1.89	0.02
Gly	GGA	3.00	0.44	0.00
Gly	GGU	365.00	52.99	0.59
Gly	GGC	238.00	34.55	0.38
Glu	GAG	108.00	15.68	0.22
Glu	GAA	394.00	57.20	0.78
Asp	GAU	149.00	21.63	0.33
Asp	GAC	298.00	43.26	0.67
Val	GUG	93.00	13.50	0.16
Val	GUA	146.00	21.20	0.26
Val	GUU	289.00	41.96	0.51
Val	GUC	38.00	5.52	0.07
Ala	GCG	161.00	23.37	0.26
Ala	GCA	173.00	25.12	0.28
Ala	GCU	212.00	30.78	0.35
Ala	GCC	62.00	9.00	0.10
Arg	AGG	1.00	0.15	0.00
Arg	AGA	0.00	0.00	0.00
Ser	AGU	9.00	1.31	0.03
Ser	AGC	71.00	10.31	0.20
Lys	AAG	111.00	16.11	0.26
Lys	AAA	320.00	46.46	0.74
Asn	AAU	19.00	2.76	0.06
Asn	AAC	274.00	39.78	0.94
Met	AUG	170.00	24.68	1.00
Ile	AUA	1.00	0.15	0.00
Ile	AUU	70.00	10.16	0.17
Ile	AUC	345.00	50.09	0.83
Thr	ACG	25.00	3.63	0.07
Thr	ACA	14.00	2.03	0.04

FIGURE 6 CONT'D

AmAcid	Codon	Number	/1000	Fraction
Thr	ACU	130.00	18.87	0.35
Thr	ACC	206.00	29.91	0.55
Trp	UGG	55.00	7.98	1.00
End	UGA	0.00	0.00	0.00
Cys	UGU	22.00	3.19	0.49
Cys	UGC	23.00	3.34	0.51
End	UAG	0.00	0.00	0.00
End	UAA	0.00	0.00	0.00
Tyr	UAU	51.00	7.40	0.25
Tyr	UAC	157.00	22.79	0.75
Leu	UUG	18.00	2.61	0.03
Leu	UUA	12.00	1.74	0.02
Phe	UUU	51.00	7.40	0.24
Phe	UUC	166.00	24.10	0.76
Ser	UCG	14.00	2.03	0.04
Ser	UCA	7.00	1.02	0.02
Ser	UCU	120.00	17.42	0.34
Ser	UCC	131.00	19.02	0.37
Arg	CGG	1.00	0.15	0.00
Arg	CGA	2.00	0.29	0.01
Arg	CGU	290.00	42.10	0.74
Arg	CGC	96.00	13.94	0.25
Gln	CAG	233.00	33.83	0.86
Gln	CAA	37.00	5.37	0.14
His	CAU	18.00	2.61	0.17
His	CAC	85.00	12.34	0.83
Leu	CUG	480.00	69.69	0.83
Leu	CUA	2.00	0.29	0.00
Leu	CUU	25.00	3.63	0.04
Leu	CUC	38.00	5.52	0.07
Pro	CCG	190.00	27.58	0.77
Pro	CCA	36.00	5.23	0.15
Pro	CCU	19.00	2.76	0.08
Pro	CCC	1.00	0.15	0.00

SEQUENCE LISTING

<110> Kavanaugh, Michael
Shyamala, Venkatakrishna
Reinhard, Christoph

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<141> 1999-06-18

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ctt cag tac tcg gag gaa gac tgt gct ttc gag gag gag atc cgc cca       194
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gat ggc tac aat gtg tac cga tcc gag aag cac cgc ctc ccg gtc tcc       242
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Pro Leu Ser His Phe Leu Pro Met Leu Pro Met Val Pro Glu Glu Pro
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His Leu Glu Ser Asp Met Phe Ser Ser Pro Leu Glu Thr Asp Ser Met
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INTERNATIONAL SEARCH REPORT

Int(ional Application No
PCT/US 00/07289

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/50 C07K16/22 A61K38/18 G01N33/68
C12Q1/68 C12N5/10 //A61P11/00,25/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NISHIMURA ET AL: "Structure and expression of a novel human FGF, FGF-19, expressed in fetal brain" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1444, 18 January 1999 (1999-01-18), pages 148-151, XP002099435 ISSN: 0006-3002 the whole document	1-13

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2000	11/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andres, S

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 00/07289

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	"zr01g05.r1 Stratagene NT2 neuronal precursor 937230 Homo sapiens cDNA clone IMAGE:650264 5', mRNA sequence" EMBL DATABASE ENTRY HS1139632; ACCESSION NUMBER AA220994 , 14 February 1997 (1997-02-14), XP002145641 the whole document & HILLIER, L. ET AL.: "Generation and analysis of 280,000 human expressed sequence tags" GENOME RES., vol. 6, 1996, pages 807-828, XP000914615 ---	1-3,5,6
P,X	WO 99 27100 A (GENENTECH INC ;BOTSTEIN DAVID (US); GODDARD AUDREY (US); GURNEY AU) 3 June 1999 (1999-06-03) the whole document -----	1-13, 19-23, 30-41

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]ional Application No
PCT/US 00/07289

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9927100 A	03-06-1999	US 6000943 A	14-12-1999
		AU 1703399 A	15-06-1999
		AU 9312198 A	05-04-1999
		WO 9914327 A	25-03-1999
		WO 9914328 A	25-03-1999